Sorption of Water Vapor and of Nitrogen by Genetic Variants of α_{s1}-Casein

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Water vapor sorption by dried preparations of α_{s1}-caseins A and C was measured at 24.2°. At lower relative pressures, both forms sorbed identical amounts of water, while at intermediate to higher relative pressures the A variant sorbed more water. A reproducible hysteresis loop was observed over the entire isotherm through two successive sorption–desorption cycles. Treatment of the H_2O sorption data and nitrogen adsorption data according to the Frenkel–Halsey–Hill isotherm equation allowed for clear distinction between the natures of the sorption processes involved with H_2O and N_2.

Introduction

It is widely accepted that proteins sorb water vapor by binding water molecules to specific hydrophilic sites at lower relative humidities followed by condensation or multimolecular adsorption as the humidity increases.[1,2] The nature of these hydrophilic sites has, however, been the subject of much research, and there is no general agreement at present concerning the chemical groupings in proteins to which water is bound.[3] The nature of these binding sites has been studied indirectly by com-

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paring the H_2O sorption capacity of proteins with that of chemically modified forms of the same proteins and more directly by using such techniques as infrared spectroscopy.[4,5]

Chemical modifications of such proteins as keratin and casein have been related to changes in water vapor sorption isotherms for these proteins. Leeder and Watt[6] used deamination techniques to demonstrate that the binding of water by amino groups constitutes a large percentage of the sorption capacity of keratin. Mellon, *et al.*,[7] found that benzoylation of amino groups reduced the water binding capacity of casein, particularly at low relative pressure. Kanagy and Cassel[8] obtained similar results with collagen after deamination, acetylation, and esterification treatments.

Though these modification techniques have been used to determine the role of particular polar groups in water binding, some investigators[6] have been concerned that these alterations of the protein by chemical reactions, particularly those which introduce new groups, may give effects other than the desired specific reaction. Therefore, more direct techniques which do not alter the protein studied are preferable.

The existence of different genetic polymorphs of α_{s1}-casein[9] has made it possible for us to compare the water vapor sorption properties of two proteins which are almost identical chemically and physiologically. These polymorphs differ only slightly in electrophoretic mobility on starch gel[10] and in amino acid composition.[11] By considering these protein forms, it is possible to study the effects of minor differences in amino acid composition of a protein upon its water vapor sorption properties without resorting to chemical modification of the protein.

This paper presents data for water vapor sorption and low-temperature nitrogen adsorption by two genetic variants of α_{s1}-casein.

Experimental Section

Samples of the genetic variants α_{s1}-casein A and α_{s1}-casein C, prepared by the method of Thompson and Kiddy,[12] were furnished by Dr. M. P. Thompson of the Eastern Regional Laboratory, U. S. Department of Agriculture, Philadelphia, Pa. Both protein preparations were dried by lyophilization.

Water vapor sorption by these proteins at 24.2° was determined gravimetrically. Samples of each of the proteins were subjected to two successive sorption–desorption cycles, and single adsorption runs were measured with additional samples of the caseins. These measurements were made using the Cahn RG recording electrobalance incorporated into a glass adsorption apparatus equipped with suitable accessories for outgassing the powders and controlling and monitoring water vapor pressure. Complete details of the apparatus and experimental technique have been presented in detail in a previous publication.[13]

Nitrogen adsorption, at −195°, was measured over the relative pressure range $0.1 < P/P_0 < 0.8$ by conventional volumetric techniques.

Results and Discussion

The water vapor sorption data yielded sigmoid type II isotherms as shown in Figures 1 and 2. Both α_{s1}-caseins displayed hysteresis loops over almost the entire relative pressure range, with the curves for the second sorption–desorption cycle essentially coinciding with those of the first cycle. The second cycle loop may be somewhat smaller for the α_{s1}-casein A (Figure 2) in the lower relative pressure range, while both loops are perfectly coincident for the α_{s1}-casein C (Figure 1). Excellent agreement was observed between data for initial sorption runs on different samples of the caseins.

The sorption legs only of these isotherms are compared in Figure 3. At lower relative pressure values, up to $0.25P_0$, the isotherms for the two α_{s1}-casein forms are almost exactly superimposable; however, at intermediate to higher relative pressures α_{s1}-casein A sorbed more H_2O than α_{s1}-casein C.

The difference in sorption capacity between the α_{s1}-caseins was not very great, yet it was a clearly reproducible difference. Rates of adsorption did not differ significantly for the two caseins, both normally requiring 8–12 hr for the attainment of equilibrium values at most points over the entire isotherm. Minimum equilibration periods of 24 hr, however, were allowed for all experimental points. Removal of sorbed H_2O after the first sorption–desorption cycle was more difficult with α_{s1}-casein A, requiring a room-temperature evacuation period of 7–10 days at 10^{-6} torr to reach the initial dry weight of the sample, while evacuation for 1–2 days sufficed with the C variant.

Studies of the amino acid composition of the genetically different α_{s1}-caseins A, B, and C by Gordon, *et al.*,[11] showed that α_{s1}-casein A is devoid of nine amino acid residues when compared with the B and C variants. Identification of proteolytic digests of the α_{s1}-caseins demonstrated[14] that eight of these amino acids were sequentially deleted from the A variant. From our

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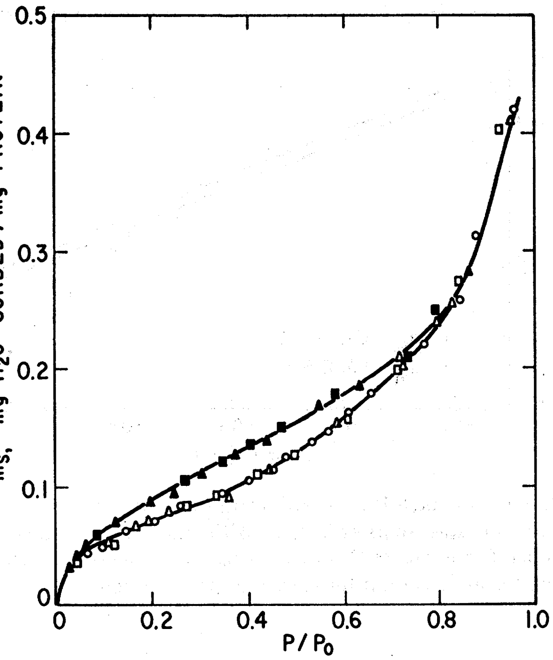

Figure 1. H_2O adsorption–desorption isotherms for α_{s1}-casein C at 24.2°: △, adsorption (first cycle); ▲, desorption (first cycle); □, adsorption (second cycle); ■, desorption (second cycle); ○, first adsorption isotherm on second sample.

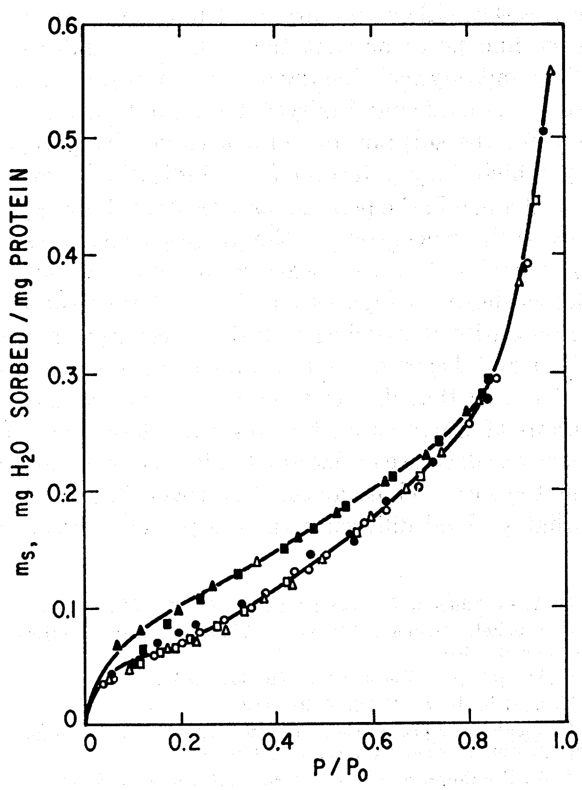

Figure 2. H_2O adsorption–desorption isotherms for α_{s1}-casein A at 24.2°: △, adsorption (first cycle); ▲, desorption (first cycle); ●, adsorption (second cycle); ■, desorption (second cycle); ○, first adsorption isotherm on second sample; □, first adsorption isotherm on third sample.

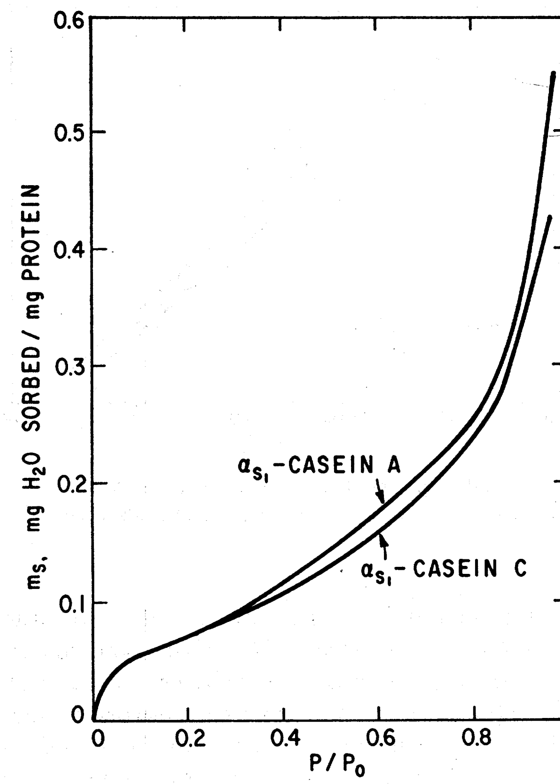

Figure 3. Comparison of H_2O adsorption isotherms for α_{s1}-caseins A and C.

data it is apparent that this peptide segment, which contains the nonpolar amino acids alanine, valine, two phenylalanines, and three leucines and the polar amino acid arginine, does not extensively affect the water binding capacity of these casein forms in the dry state. Thompson, *et al.*,[15] however, reported that the loss of this segment of the molecule has profoundly affected its solubility in aqueous $CaCl_2$ solutions. At temperatures of 10–33°, α_{s1}-casein A remains soluble under conditions in which the B and C variants are totally insoluble. Thompson, *et al.*,[15] suggested that the increased solubility of the A variant may be due to less extensive hydrophobic bonding and/or altered protein conformation in solution in the absence of these principally nonpolar amino acid residues. The data reported in this paper together with their solubility data, however, lead to the conclusion that this hydrophobic peptide segment as present α_{s1}-casein C must have a conformation on the surface of the molecule which influences its overall solubility.

There has been wide discussion in the literature[1,16] concerning the applicability of the BET equation[17] to

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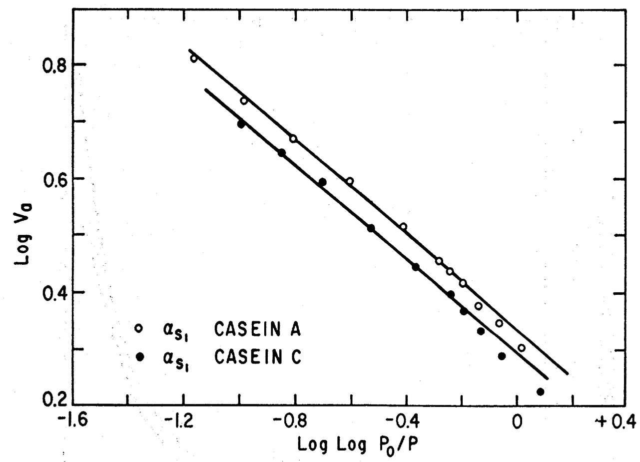

Figure 4. Frenkel–Halsey–Hill isotherm plots for N_2 adsorption at $-195°$ on α_{s1}-caseins (V_a, volume adsorbed as cm^3/g).

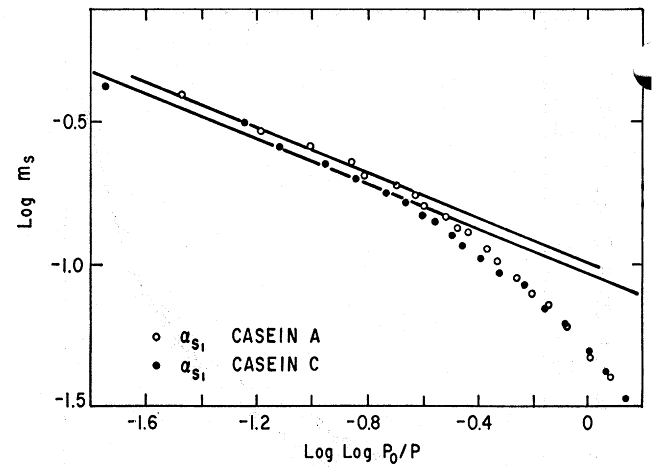

Figure 5. Frenkel–Halsey–Hill isotherm plots for H_2O vapor sorption on α_{s1}-caseins at 24.2°. Although similar results were obtained for all the H_2O sorption runs, only one set of data is shown for each variant for clarity in presentation.

the sorption of water vapor by dried proteins or other materials of biological origin. It has been pointed out[18] that the significance of a BET monolayer with regard to vapor sorption in a swelling polymer network is questionable. Treatment of our data for H_2O and N_2 adsorption according to the isotherm equation of Frenkel,[19] Halsey[20], and Hill[21] (FHH) yielded information distinguishing between the sorption processes involved.

Data obtained from N_2 adsorption measurements yielded linear plots, as shown in Figure 4, in the relative pressure range predicted by Pierce[22] for physical adsorption on surfaces with no unusual capillarity. It has been previously demonstrated that N_2 adsorption data for high polymers[23] and dried material of biological origin[24] do yield linear plots with the FHH isotherm equation. The plots in this paper have steeper slopes than the standard plot for nitrogen adsorption of Pierce.[22] Hightower and Emmett[23] made similar observations with polyolefins. They attributed these deviations in slope to lowered heats of adsorption for N_2 as evidenced by the low C values, ranging from 11 to 48, which they obtained from their BET plots. We obtained a low C value of 32 for N_2 adsorption on α_{s1}-casein C; however, the α_{s1}-casein A data yielded a C value of 79.

When our water sorption data were plotted according to the FHH equation, wide deviations from linearity were observed, as shown in Figure 5. The linear portion of the curves shown in this figure correspond to a value of $r = 2.5$ in the FHH equation

$$\log \log (P_0/P) = r \log \theta$$

where θ corresponds to the fraction of surface coverage or any variable proportional to it such as volume or mass adsorbed. The value of 2.5 for the constant r is in agreement with that observed by Halsey[20] for the adsorption of H_2O on anatase at 25°. According to Pierce,[22] such a value of $r = 2.5$ should be standard for adsorption of water vapor on any adsorbent where strictly multilayer adsorption is occurring.

The linear portion of the FHH isotherm shown in the present paper can correspond to multilayer adsorption at higher relative pressures. However, the curved nature of the major portion of these FHH isotherm indicates that either capillary condensation is occurring in very fine pores or that the sorbent is undergoing swelling and physical changes as a result of the sorption process. Brandt and Budrys[25] have published similar curves for the sorption of various vapors by polypeptides, which they interpreted as indicating morphological changes in the peptides as a result of the sorption.

Any swelling occurring in the α_{s1}-caseins is, however, fully reversible through at least two sorption–desorption cycles as shown in Figures 1 and 2. These results are not necessarily at variance with those recently reported by Rao and Das[26] for native and denatured caseins. They showed that during a series of sorptions and desorptions of water vapor the hysteresis loop showed a tendency to decrease in size and finally disappear in subsequent cycles. Those authors, however, have pointed out that varietal differences in the proteins affect the

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tendency of the hysteresis loop to disappear in successive sorption–desorption cycles.

Nevertheless, the sharp contrast between the shapes of the FHH plots for N_2 and H_2O sorption of the α_{s1}-caseins further demonstrates the basic differences in the types of interactions involved in H_2O and N_2 adsorption on dried proteins.

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